| **Friday, February 24, 2006** | |
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| **USDA CSREES - NRI Functional Genomics Project Directors Meeting**<br>Presentations: 8:00am – 12:00pm<br>Room: Waterway 6<br>Breakout Session: 1:00pm – 4:00pm<br>Room: Waterway 6, 7 & 8 | |
| 7:45am – 8:00am | **Load Powerpoint Presentations!** |
| 8:00am – 8:15am | **COFFEE** |
| 8:15am – 8:20am | Ed Kaleikau, USDA-CSREES<br>**Welcome and Introduction** |
| 8:20am – 8:30am | Anna McClung, USDA-ARS<br>**USDA-ARS Rice Seed Stock Center: Genetic Stocks – Oryza (GSOR) Collection** |
| **New 2005 PROJECTS** | |
| 8:30am – 8:40am | C. Robin Buell, The Institute for Genomic Research<br>**Rice SNP Project: Sequencing Multiple and Diverse Rice Varieties to Allow Connection of Whole Genome Variation With Phenotype** |
| 8:40am – 8:50am | Jiming Jiang, University of Wisconsin<br>**Identification, Transcription and Epigenetic Modification of Genes in Rice Centromeres** |
| 8:50am – 9:00am | Benildo de los Reyes, University of Maine<br>**Cold Stress Response Gene Regulon in Rice** |
| 9:00am – 9:10am | Naohiro Kato, Louisiana State University<br>**Technology Development: *in situ* High-Throughput Analysis of Protein-Protein Interactions for Cereals** |
| 9:10am – 9:20am | Ning Jiang, Michigan State University<br>**The Impact of Rice *Mutator*-like Elements on Gene Expression and Gene Function** |
| 9:20am – 9:30am | Pam Ronald, University of California<br>**Elucidating the NRR/NH1 Mediated Resistance Signaling Network** |
| 9:30am – 9:40am | Venkatasen Sundaresan, University of California<br>**Sequenced Insertion Lines for Rice Functional Genomics** |
| 9:40am – 10:00am | **BREAK** |
| **2004 PROJECTS** | |
| 10:00am – 10:15am | Blake Meyers, University of Delaware<br>**Deep Profiling of Small Rice RNAs by MPSS** |
| 10:15am – 10:30am | Zhaohua Peng, Mississippi State University<br>**Proteome Analysis of Chromatin Associated Proteins During Endosperm Development in Rice (*Oryza sativa*)** |
| 10:30am – 10:45am | David Galbraith, University of Arizona<br>**Microarray Based QTL Mapping in Rice** |

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| **2003 PROJECTS** | |
| 10:45am – 11:05am | Dick McCombie, Cold Spring Harbor Laboratory<br>**Systematic Determination of the Rice Gene Set** |
| 11:05am – 11:25am | Guo-Liang Wang, Ohio State University<br>**Use of Oligo Arrays to Dissect Rice Defense Response Pathways** |
| 11:25am – 11:45am | Luca Comai, University of Washington<br>**Tilling Resources for *Japonica* and *Indica* Rice** |
| 11:45am – 12:00pm | **WRAP UP – Ed Kaleikau**<br>**Breakout Discussion Points and Logistics:**<br>**After lunch we will hear a presentation from Hei Leung about the IRFGC then we will breakout into 3 groups. Venkatasen Sundaresan, Blake Meyers, and Luca Comai have graciously agreed to serve as group discussion facilitators. Each group will meet separately and discuss the questions listed below. We will reconvene and each group has an opportunity to report out. Hei Leung has kindly agreed to draft a summary report of our discussion (with the help of the group facilitator notes!).** |
| 12:00pm – 1:00pm | **LUNCH** |
| **AFTERNOON BREAKOUT SESSIONS** | |
| 1:00pm – 1:05pm | Ed Kaleikau, USDA-CSREES<br>**Introduction** |
| 1:05pm – 1:25pm | Hei Leung, International Rice Research Institute<br>**International Rice Functional Genomics Consortium** |
| 1:30pm – 3:00pm | **Breakout Groups Meet – Waterway Rooms 6, 7 & 8**<br>**Facilitators:**<br>**Group 1: Venkatasen Sundaresan**<br>**Group 2: Blake Meyers**<br>**Group 3: Luca Comai**<br>1. What are the knowledge gaps and research, education and extension opportunities in rice functional genomics?<br>2. How can we move the field of rice functional genomics forward to have the greatest impact? In other words, what functional genomics research, education and extension is needed to provide the best return on investment to the U.S. taxpayer?<br>3. What would a broad road map of this research, education and extension plan look like? |
| 3:00pm – 4:00pm | **RECONVENE AND REPORT OUT TO THE WHOLE GROUP - Waterway Room 6, 7, or 8** |
| 4:00pm | **ADJOURN TO THE BAR TO CONTINUE DISCUSSION** |
| 6:30pm | **DINNER** |

Cooperative State
Research, Education and Extension Service

# PROGRAM ABSTRACTS
# USDA CSREES - NRI Functional Genomics Project Directors Meeting

## USDA-CSREES NRI PLANT GENOMICS, GENETICS AND BREEDING

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This USDA CSREES NRI Plant Genome Program supports research, education and extension projects ranging from technology development to fundamental science and practical application for U.S. crop or forestry improvement. The priorities focus on technological advances and discoveries in areas such as a) analytical methods for mapping genes for complex traits for direct use by plant breeders, b) novel methods for analysis of the genome and its effect on biological function, c) cost-effective sequencing strategies to understand complex genome structure and organization, d) procedures to analyze the total expression patterns of genes under specific conditions, and e) appropriate data handling and analysis capabilities. The ultimate goal of the program is to contribute knowledge about the biology of agriculturally important plant processes and traits, which can be used to develop crops with enhanced economic value and expanded utilities.

To meet these identified needs of agriculture, the long-term goals are: increased fundamental knowledge of the structure, function and organization of plant genomes to improve agricultural efficiency and sustainability; effective integration of modern molecular breeding technologies and traditional breeding practice for U.S. crop and forestry improvement; and improved U.S. varieties for agricultural growers and producers.

The program coordinates its goals with the Interagency Working Group on Plant Genomes and the participating agencies (NSF, DOE, NIH, etc). Since 1999, CSREES has provided nearly $20M of funding for rice genomics, genetics and breeding. Activities have included support for genome sequencing (IRFGC), comparative informatics (Gramene), functional genomics (IRFGC), translational genomics (RiceCAP), proteomics and metabolomics. The program focuses on agriculturally significant plant families or species in four elements: (1) Tools, Genetic Resources and Bioinformatics, (2) Functional Genomics, (3) Genome Structure and Organization and (4) Integrated Applied Plant Genomics Coordinated Agricultural Projects.

Acknowledgement: CSREES is the USDA's major extramural research agency, funding both individuals and institutions. NRI grants enable researchers, educators and extension specialists throughout the United States and internationally to solve problems critical to farmers, consumers, and communities.

**GENETIC STOCKS – Oryza (GSOR) COLLECTION**

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In August, 2003, the Genetic Stocks - Oryza (GSOR) Collection was established at the USDA-ARS Dale Bumpers National Rice Research Center, at Stuttgart, AR. The center has a dedicated laboratory, greenhouse and field space for seed increase and evaluation, and 4°C cold room for seed storage. A protocol for donating stocks to the center has been established and requires information on origin and/or pedigree, a phytosanitary certificate, and permission for public distribution free of intellectual property rights.

Currently the GSOR collection includes 20 accessions and two mapping populations. Various genetic mutants are available including early fowering, semidwarfism, brittle, male sterile, gold hull, lesion memic, and elongated upper internode. A set of 191 mutants, donated by Japan's Dr. Toshiro Kinoshita, via Dr. Susan McCouch, Cornell University, has been approved for entry to the GSOR collection. This material arrived with complete gene characterization and chromosomal identification and is currently undergoing seed increase before distribution can occur. In addition, giant embryo apoptosis, chives, extreme dwarf, and gold leaf mutants develop by Dr. Rutger will be deposited in the collection in 2006. The mapping populations at GSOR currently include 353 RILs from a Kaybonnet Ipal/Zhe 733 and 325 double haploid lines from Cocodrie/MCR01-0277. The latter population was developed by Dr. Qi Ren Chu at Louisiana State University and is one of the mapping populations being used to identify QTL for sheath blight resistance in the RiceCAP project.

In January, 2005, the GSOR became a distribution site for Nipponbare. The accession was donated to the collection via Dr. Susan McCouch, Cornell University, and is a direct descendent of the selection Dr. T. Sasaki of Japan that was used in the International Rice Genome Sequencing Project.

The GSOR website (**http://ars.usda.gov/Main/docs.htm?docid=8318)** lists all of the accessions and information on donating or obtaining the stocks.

# SEQUENCING MULTIPLE AND DIVERSE RICE VARIETIES TO ALLOW CONNECTION OF WHOLE-GENOME VARIATION WITH PHENOTYPE

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The International Rice Functional Genomics Consortium (IRFGC) has initiated a project to identify genome sequence diversity within rice germplasm using a high throughput hybridization-based sequencing method. In parallel, we will characterize this set of germplasm to provide phenotypic data for rice biologists. In this project, we will collaborate with the International Rice Functional Genomics Consortium and with Perlegen Sciences to identify a large fraction of the single nucleotide polymorphisms (SNPs) present in cultivated rice through whole-genome comparisons of 21 rice genomes, including cultivars, germplasm lines, and land races. SNPs will be available to the public through intuitive web interfaces via the project website (http://oryzasnp.org) which will allow data-mining of the polymorphisms in a genomic context. Query and search pages will also be made available. The SNP data will be tied to the rice genome annotation thereby allowing users to determine the impact of a polymorphism at the gene/protein level. To determine the phenotypic variation within the 21 rice lines nominated for resequencing, we will evaluate more than 33 traits in five broad categories, i.e., (1) reproductive and yield-related, (2) morphological, (3) grain quality, (4) abiotic stresses, and (5) biotic stresses. The phenotypic data will be integrated into the International Rice Information System database (http://www.iris.irri.org).

Acknowledgments:
This project is supported by a National Research Initiative Competitive Grant 2006-35604-16628 from the USDA Cooperative State Research, Education, and Extension Service.

# IDENTIFICATION, TRANSCRIPTION AND EPIGENETIC MODIFICATION OF GENES IN RICE CENTROMERES

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The goal of this proposal is to identify and characterize all active genes located within the centromeres of rice chromosomes. The centromere has traditionally been viewed as a genetically silent and highly heterochromatic chromosomal domain. The centromeres of most model eukaryotic species, including humans, *Drosophila melanogaster*, and *Arabidopsis thaliana*, are embedded within cytologically distinctive heterochromatin and are composed of highly repetitive DNA sequences. Genetic recombination in the centromeric regions is severely suppressed in these species. Therefore, the centromeres of these model species represent the classically defined centromeres. However, recent studies on rice centromeres have dramatically challenged this traditional concept. Our lab has sequenced the entire centromere of rice chromosome 8 (CEN8). A total of 16 active genes were discovered within the 750-kb functional domain of CEN8. The recombination-suppressed domain of CEN8 spans 2,312 kb and includes at least 86 active genes.

The specific goals of this proposal include: **(1)** to annotate sequences associated with all 12 genetically defined rice centromeres that are free of recombination; **(2)** to profile the transcription of the centromeric genes using genomic DNA tiling arrays; **(3)** to profile the histone modification patterns associated with the centromeric genes using a ChIP-on-chip approach. The proposed research will catalogue all of the genes in rice centromeres. This collection of "centromeric genes" will be a foundation to study structure, function and evolution of genes located within recombination-free domains and to understand the functional mechanisms of rice centromeres. The data generated from this project will be publicly available using an existing, popular, and user-friendly web interface maintained at www.hort.wisc.edu/faculty/jiang.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2006-35604-16649 from the USDA Cooperative State Research, Education, and Extension Service.

**COLD STRESS RESPONSE GENE REGULON IN RICE**

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Direct-seeded rice is highly prone to low temperature stress during the early stages of seedling establishment. In general, japonicas are more tolerant than most indicas. The biochemical and physiological bases of tolerance mechanisms involve an integrative functioning of a large number of genes that operate through a highly coordinated regulatory network.

This research will dissect the cold stress response regulatory pathways of japonica rice by reconstructing the hierarchical organization of the various '*regulator-effector*' modules of gene expression (transcription factor and downstream targets) associated with adaptive responses. First, we will define the critical biochemical processes involved in cold tolerance mechanisms based on the global patterns of gene expression in two japonica cultivars (CT6748-8-CA-17, Nipponbare) with comparable levels of cold tolerance at seedling stage. The cold stress transcriptome will be profiled at 10 time points during the most critical stage of stress (first 96-hrs) and at 72-hrs post-stress recovery period using the whole-genome oligonucleotide microarray from the NSF-Rice Oligonucleotide Array Project (www.ricearray.org). Second, clusters of co-regulated genes will be established based on coordinated patterns of expression. This information will be used to identify all the cis-elements that define the different branches of the regulon by *in silico* analysis of the promoters of co-regulated genes using the Nipponbare genome as a model. The results will be validated by cross-species comparison and phylogenetic footprinting with orthologous genes from closely related cereals (maize and sorghum). Critical promoter elements will be validated experimentally by promoter-GUS expression assay in transgenic plants. Promoter deletion and mutation studies will be performed to determine the various elements that interact with the major regulators of the cold stress response genetic circuit and to assess the complexity of the transcriptional machinery. Candidate regulators for each critical element will be deduced by quantitative RT-PCR of corresponding '*regulator-effector*' modules in cold-tolerant and intolerant rice genotypes. Further functional validation will be performed by anchoring the candidate regulatory genes to the established molecular genetic maps of cereals with respect to the known cold tolerance-associated QTL using Gramene (www.gramene.org) as the platform.

This research will establish a database of all regulatory genes and downstream target clusters involved in cold tolerance mechanisms. The knowledge generated will serve as a foundation for future investigation of the signaling network by reverse genetics. Understanding the hierarchical organization of such a network could impact rice breeding through the identification of the key genes and/or pathways for targeted selection or manipulation. The study will also contribute to the discovery of novel stress-regulated promoters for various applications in rice functional genomics and biotechnology.

This project represents a collaboration between the University of Maine (Benildo G. de los Reyes) and IRRI (Richard Bruskiewich). IRRI will provide additional capabilities for microarray data analysis and genome sequence mining through a short-term shuttle research program. All information (microarray and promoter data) will be disseminated through Gramene and NCBI (Gene Expression Omnibus).

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2006-35604-16693 from the USDA Cooperative State Research, Education, and Extension Service.

**TECHNOLOGY DEVELOPMENT: *in situ* HIGH-THROUGHPUT ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS FOR CEREALS**

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The goal of this project is to establish high-throughput *in situ* (the original place) protein-protein interaction (PPI) assay systems for cereals. We will 1) construct a series of plasmids and 2) optimize *in situ* PPI detection systems. In addition to using the FRET (fluorescence resonance energy transfer) method for PPI detections at subcellular levels, we will apply the split-luciferase method to study the PPI dynamics at tissue levels for a large-scale analysis.

The plasmids we developed allow rapid construction of a dual-gene-expression cassette through Gateway® and Cre-loxP *in vitro* recombination reactions. Different tags are translationally fused to each gene product so that PPI between the two gene products can be analyzed in living cells. The sequence of the promoters and the tags in the plasmids can be replaced using unique restriction enzymes so that one can customize the plasmid for organisms of their interest.

Our objectives are:
1. Construct the plasmids for high-throughput *in situ* protein-protein interaction analysis for cereals.
2. Conduct proof-of-concept experiments using selected protein-pairs in rice.

This project will provide molecular tools and protocols to the rice community. We will test several protein-pairs to optimize the assay conditions.

The plasmids will be publicly available through the PD laboratory upon request immediately after the proper function in rice is confirmed. The protocols will be posted on the PD's website.

The project will be conducted as a two-year pilot project at Louisiana State University.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2006-35640-16627 from the USDA Cooperative State Research, Education, and Extension Service.

**THE IMPACT OF RICE MUTATOR-LIKE ELEMENTS ON GENE EXPRESSION AND GENE FUNCTION**

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The goal of this project is to elucidate the possible role of Mutator-like elements (MULEs) on the regulation of downstream genes as well as the generation of new functional coding sequences. MULEs are very abundant in rice and a subset of them (over 3000 copies) carries recognizable host genes or gene fragments, which are called Pack-MULEs. Previous studies have shown that those elements preferentially inserted into regulatory regions. To test the effect of MULEs on the expression of downstream genes, polymorphic insertions of MULEs between the two sequenced cultivars, Nipponbare and 93-11, will be identified through computational approaches. Thereafter, the transcripts of the relevant genes in the two cultivars will be detected through RNA blot or RT-PCR. For those loci that display differential expression, the transcription start site will be defined, methylation status of the relevant promoter region will be tested, and control experiments will be performed to determine whether there is a casual relationship between the MULE insertion and the expression of downstream genes. Our preliminary data indicated that MULEs alternated the tissue specificity of some genes.

Another interesting feature of MULEs is the acquisition of genomic sequences including gene fragments. Given the abundance of these elements, it was reasoned whether some of them may evolve into functional genes. To test this notion, RNAi constructs based on the element sequences will be made and transformed to rice in order to knock out some of the Pack-MULEs. In this case, it is possible to evaluate whether the Pack-MULE is responsible for any phenotypic change or the alternated expression of downstream genes.

Our objectives are:

1. Identify 50 loci where the MULE insertion are polymorphic between NIpponbare and 93-11 and test the expression of downstream genes.
2. Test the function of a Pack-MULE-CBF gene and its relationship with endogenous CBF genes.

This project will provide a list of the polymorphic loci between Nipponbare and 93-11 as well as the relevant expression data. The results from functional analysis of the Pack-MULE-CBF gene will be available in publication, if it is functional.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2006-35604-16631 from the USDA Cooperative State Research, Education, and Extension Service.

**ELUCIDATING THE NRR/NH1-MEDIATED RESISTANCE SIGNALING NETWORK**

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We have established that the rice proteins NH1 and NRR are key mediators of disease resistance. Over-expression of *NH1* (NH1ox) enhances resistance and over-expression of *NRR* (NRRox) enhances susceptibility to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a serious disease of rice worldwide. *NH1-OX* rice plants also display enhanced cell death when grown in a low-light environment or treated with the chemical BTH. Using a hi-throughput yeast two-hybrid approach, we have generated a protein interaction network consisting of 22 proteins, of which five have been confirmed by functional approaches to be involved in this pathway

The long-term goals of this proposal are to further elucidate the signal cascade governing disease resistance in monocot species and to create novel strategies for disease control in rice and other cereal crops. The short-term objectives are to expand our knowledge of the NRR/NH1-mediated resistance signaling network by using genetics and proteomic approaches. To accomplish this objective we will (1) Characterize mutants of candidate genes in the network to determine the functional importance of these genes in defense response signaling (2) Characterize the cellular interactions of NRR, NH1 and TGAs to confirm biological relevance, determine hierarchy of interaction and identify new signaling proteins (3) Identify new mutants in the NRR/NH1 signaling pathway that are suppressed for the cell death and defense response phenotypes.

The results from the project will contribute to the long-term goals of the International Rice Functional Genomics Consortium to determine the function of all the rice genes within 10 years, and to identify genes that make rice different from Arabidopsis.

The data will be publicly available using an existing, popular, and user-friendly web interface http://rkd.ucdavis.edu/, allowing plant biologists to determine if their gene(s) of interest is part of the innate immunity interaction network. The website will integrate these data with mRNA profiling data from our NSF supported microarray project.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2005-35604-16640 from the USDA Cooperative State Research, Education, and Extension Service.

**SEQUENCED INSERTION LINES FOR RICE FUNCTIONAL GENOMICS**

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With the completion of the rice genome sequence, insertional mutants that disrupt gene function will be critical for assigning biological functions to genes. The International Rice Functional Genomics Consortium (IRFGC), which includes the PD and co-PD, has identified as a major scientific goal the saturation mutagenesis of the rice genome using insertion elements. We have developed a novel system of fluorescent markers for the selection of stable transposon insertions, and have demonstrated that the maize *En/Spm* transposons act as very efficient insertional mutagens in rice. To date, we have generated ~10,000 *Ds* and *dSpm* insertion lines in the sequenced cultivar Nipponbare, of which ~6,000 have been sequenced. At the end of the project, we will have available a collection of publicly available seed stocks for ~35,000 insertion lines, with genomic locations for each insertion determined by DNA sequencing. The sequenced insertion sites are available for searches in a publicly accessible database at UC Davis (http://sundarlab.ucdavis.edu/) and in Genbank, and are also displayed in the rice genome browsers at the TIGR and Gramene sites.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2006-35604-16674 from the USDA Cooperative State Research, Education, and Extension Service.

**DEEP PROFILING OF SMALL RICE RNAs BY MPSS**

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The goal of this proposal is to use novel methods for the isolation and characterization of small RNA molecules (21 to 24 nucleotides) from the crop plant rice (*Oryza sativa*). These molecules will be sequenced using a method called massively parallel signature sequencing ("MPSS"). These molecules represent endogenous small interfering RNAs (siRNAs) or microRNAs (miRNAs); these RNAs have specific biological activities in many eukaryotes. The relative abundance of distinct sequences in each library provides quantitative expression data, while comparisons of these sequences to genomic DNA may identify the source and/or target of these RNA fragments.

This project will result in a collection of more than three million small RNA sequences from 11 libraries. In total, this data set will represent the most extensive collection of small RNA sequences available for any organism. The data that we are developing will be broadly useful and applicable as a public resource and will identify and measure small RNAs that are regulated in response to developmental, biotic or abiotic cues. The biological relevance of small RNAs that are identified through this project can be assessed by individual investigators. Comparisons of small RNA abundances across tissues and treatments will provide novel insight into the regulation of these molecules under diverse developmental or stressed conditions.

The major goal of this project is to develop a rice small RNA resource based on the identification of small RNA molecules in the following samples:

- Diverse wild-type rice tissues.
- Abiotically-stressed rice tissues.
- Resistant and susceptible interactions in rice to the rice blast fungus (*Magnaporthe grisea*).

The data will be publicly available using an existing, popular, and user-friendly web interface http://mpss.udel.edu/rice, allowing plant biologists to determine if their gene(s) of interest is targeted by small RNAs. The website will integrate these data with mRNA profiling data from conventional MPSS.

The project represents collaboration between the laboratories of Drs. Blake Meyers and Pam Green at the University of Delaware. The laboratory experiments are being conducted in Dr. Green's lab by post-doctoral scientist Dr. Cheng Lu with the assistance of a technician, Rana German. The bioinformatics work is performed in Dr. Meyers's lab by Karthik Kulkarni, a master's degree student from the Department of Computer Science, with assistance from other bioinformatics students (Kalyan Vemeraju and Jeremy Skogen). The development and implementation of small RNA tools on our website is lead by Mayumi Nakano.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2005-35604-15326 from the USDA Cooperative State Research, Education, and Extension Service.

# PROTEOME ANALYSIS OF CHROMATIN ASSOCIATED PROTEINS DURING ENDOSPERM DEVELOPMENT IN RICE (*Oryza sativa*)

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Endosperm is a unique organ with three sets of genomes in its cell in higher plants. Chromatin mediated gene regulation plays an essential role in endosperm development, including imprinting, ploidy barrier of hybridization, gene dosage effect, and endoreduplications. Understanding the underlying molecular mechanism will shed new light on chromatin mediated developmental regulation and probably lead to new avenue for crop improvement, including stabilizing hybrid vigor for example. A critical step in the study of endosperm chromatin is to purify the chromatin proteins and identify its unique feature(s) compared to the chromatin of other organs and tissues. Unfortunately, the majority of cells in endosperm are highly rich in starch grains and storage proteins, making the purification in large scale extremely challenge. We have successfully established a reliable protocol to purify high quality chromatin from rice (*Oryza sativa*) endosperm in large scale. The protocol includes extraction of nuclei from developing endosperm, purification of the nuclei, purification of chromatin from nuclei, and extraction of chromatin proteins. Meanwhile, we have also obtained highly purified chromatin proteins from rice suspension culture cells. Mass spectrometry analysis of the purified chromatin proteins are in progress. In addition, we have developed MUDPIT (Multidimensional Protein Identification Technology) for mass analysis and genome ontology to explore protein function. Chromatin proteins are highly basic. The MUDPIT method has many advantages over the traditional 2-D gel based methods. Finally, we have identified 13 rice knockout mutant lines of the genes encoding chromatin proteins with potential roles in endosperm development in rice Tos17 insertion libraries and made 12 RNAi constructs. Transgenic lines of 4 RNAi constructs have been generated in rice.

Acknowledgment:
This project is supported by a National Research Initiative Competitive Grant 2005-35604-15357 from the USDA Cooperative State Research, Education, and Extension Service.

**MICROARRAY BASED QTL MAPPING IN RICE**

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The goal of this project is to develop microarray platforms for Quantitative Trait Locus (QTL) mapping in rice. Microarrays will be applied in both the genotyping and expression profiling of QTL mapping populations. The high resolution genotyping and gene expression data acquired through this project will be combined with phenotypic information to map and dissect QTLs of agronomic importance.

The first objective of this project is to develop a microarray technology for rapid and cost-effective genetic mapping. Our genotyping microarray technology uses 70mer oligonucleotide probes spotted on slides to detect Single-Feature Polymorphisms (SFPs) (i.e. insertions/deletions) in hybridized fluorescently labeled target DNA. We have identified over 1000 SFPs suitable for probe design by aligning the publicly available genomic sequences of the Nipponbare and 9311 cultivars representing the *japonica* and *indica* sub-species of rice. We have printed a subset of 500 oligonucleotide probes with good genomic distribution and verified the polymorphisms using hybridizations with DNA from the Nipponbare and 9311 reference genotypes. The genotyping microarrays are being tested using a collection of diverse rice cultivars to further assess the levels of polymorphism. Once optimized, genotyping arrays will be used for QTL mapping in the IR64 X Azucena doubled haploid population and the SHZ X LTH recombinant inbred line population. The genotyping data will be integrated into the existing molecular maps of these segregating populations to facilitate high resolution QTL mapping.

In the next phase, we will implement a “genetical genomics” approach using microarrays for expression profiling of all individuals in the SHZ X LTH mapping population. Integrating the gene expression data with the mapping data will allow dissection of each transcript profile into its *cis*-acting and *trans*-acting genetic components. This analysis is expected to provide information about gene networks and yield candidates for identifying genes underlying the QTLs.

This project will deliver microarray tools and populated databases to the US and international rice/plant science communities, promoting greater connectivity and accelerating research. These contributions will be complemented by microarray training workshops at the University of Arizona. The laboratory of Dr. David Galbraith will act as the location for microarray platform development, genotyping, and expression profiling experiments, with post-doctoral scientists Dr. Jeremy Edwards, Dr. Ambika Gaikwad, and Dr. Jaroslav Janda, research specialist Georgina Lambert, and visiting student Stefan Schwab. The DNA and RNA samples are being obtained from the International Rice Research Institute (IRRI) with the assistance of the Cooperating PD, Dr. Hei Leung. A program of exchange visits has been initiated to efficiently allow technology and biomaterials transfer between the project sites.

Acknowledgment:
This project is supported by a National Research Initiative grant 2005-35604-15327 from the USDA Cooperative State Research, Education, and Extension Service.

**SYSTEMATIC DETERMINATION OF THE RICE GENE SET**

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Aside from a small number of ESTs / mRNAs from other sources, the Riken rice cDNA collection represents the bulk of the known expressed sequences in rice. However, the quality of this data set has not been evaluated. Presented here is a directed evaluation of the 5' end of a number of Riken rice cDNAs to determine the completeness of the mapped gene model. Based on Riken cDNA sequences, an optimized, high-throughput RACE procedure was used to successfully amplify the 5' ends of 424 genes from rice leaf RNA. Comparison of the Riken cDNA and the RACE sequences revealed that while 27.2 % of the RACE sequences contained putative transcriptional start sites (TSSs) similar to the Riken cDNA's, the remaining 72.8% of RACE transcripts were either completely different or had some forms that were different. These different TSSs identified represent 490 novel putative TSSs. Additionally, 17% of the RACE transcripts showed internal exons serving as alternate 1$^{st}$ exons. Nearly 8% of the RACE transcripts contained novel exons not represented in the Riken cDNA while 7% of the RACE transcripts contained novel 1$^{st}$ exons. In addition, multiple splice isoforms were identified in >20% of the RACE transcripts, which indicates that splice variation may exist at a higher level in plants than previously thought. These results indicate that there are significant amounts of variation present in rice genes that are not currently represented.

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# USE OF OLIGO ARRAYS TO DISSECT RICE DEFENSE RESPONSE PATHWAYS

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The goal of this proposal is to use rice oligo arrays to rapidly and efficiently classify defense mutants in rice. Ten carefully selected mutant lines that exhibit altered defense responses to two pathogens were profiled before and after fungal (*Magnaporthe grisea, Mg*) and bacterial blight (*Xanthamonas oryzae* pv. *oryzae, Xoo*) infections. Based on their expression patterns, the mutants have been classified into several groups and a set of novel defense-related genes have been identified. We will use this information to generate transgenic lines with enhanced resistance. The key findings obtained in 2005 are summarized below:

- *Mg* and *Xoo* inoculations were performed on all the mutant plants grown in the same growth chamber conditions. RNA was isolated from the inoculated and control plants at two- and eight-week stages. Purified mRNA samples were shipped to the Ronald lab for microarray hybridizations with the 21K NSF-funded rice oligo arrays. The protocols for dye labeling, hybridization and imaging scanning were optimized.

- A total of 132 hybridizations were performed in the Ronald lab. Among them, 106 hybridizations were successful. Using the R Language, LMgene and Partek programs, the dataset was extensively analyzed. Quality test of the data indicated that the hybridizations were highly reproducible and the major source of variation was from the treatments.

- Comparative analysis of the expression profiles performed in the Ronald lab revealed significant differences among the tested mutants. Mutants in the JA and SA pathways exhibited no overlapping up or down-regulated genes with the other mutants tested. Lesion mimic mutants *spl11* and *spl17* showed a highly similar expression patterns before and after pathogen inoculations. Many genes were commonly expressed in the *Xa21* and *NRR1ox* plants after *Xoo* inoculation. Novel genes specifically induced or repressed in the *NRR1ox* and *NH1ox* plants were also identified.

- To accelerate gene discovery in untagged mutations, the Leach lab is optimizing an array-based deletion detection method. A cluster of deletions in radiation mutants (*spl1-2* and *spl1-3*) was detected on chromosome 12 where the *Spl1* gene is located. Mutations in three candidate genes were confirmed by PCR method. RNAi and BMV-VIGS strategies are being used to pinpoint the targeted gene.

- The Leung lab at IRRI has produced over 70 double mutants and evaluated 50 double mutants for bacterial blight resistance and lesion mimic appearance. Homozygous double mutants for some the pairs have been obtained and will be provided to the project for the second hybridization in the end of 2006.

- The Ronald and Wang labs established an efficient rice protoplast-based transient expression assay for rapid defense gene characterization in rice. The GFP/LUC reporter constructs of *PBZ1, PR1* and chitinase genes were successfully tested in the Wang lab. The Ronald lab has shown that genes can be silenced by transfecting siRNA constructs into the protoplasts. The established method can be used for large-scale screening of candidate defense genes from our microarray analysis when their over-expression or RNAi construct is co-transfected with a defense gene reporter construct in rice protoplasts.

- The project website (www.ricedefensemutant.org) has been constructed and is now linked to the NSF supported microarray web site (www.ricearray.org). The microarray hybridization images and preliminary analysis results will be publicly available after the completion of the quality check of the whole dataset.

- An outreach program, “Classroom Activities in Plant Biotechnology”, was developed at CSU. In 2005, lectures on the importance of food crops in our lives and the problems that will occur in food production were given to about 520 children in several elementary and middle schools in Colorado. Many of the students are from Hispanic families.

- The project represents a close collaboration between the laboratories of Guo-Liang Wang, Pam Ronald, Jan Leach and Hei Leung. The leaf tissue harvesting and RNA isolation were performed at OSU, microarray hybridizations and image processing at UCD, deletion detection of *spl1* mutant and biotechnology outreach at CSU, double mutant generation at IRRI. Students and postdocs from all labs have participated in microarray data analysis

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**TILLING RESOURCES FOR JAPONICA AND INDICA RICE**

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The goal of this proposal is to produce a TILLING–based reverse genetics resource for rice (*Oryza sativa*). Research in this project will define optimal conditions for standard chemical mutagenesis of rice. Mutagenesis efficiency will be monitored by the use of phenotypic markers and by molecular genotyping at selected loci to identify point mutations through PCR amplification of pooled DNAs followed by the use of the mismatch-detecting CELI and resolution of cut products on Li-cor DNA analyzers. This technology, called TILLING, is already developed and has been proven with Arabidopsis, maize, wheat, zebrafish etc. Suitably mutagenized rice populations will be inventoried and stored and their DNAs used in TILLING screens. A number of genes will be selected and existing informatics tools will be used to develop TILLING primers. Informatics tools have been developed to select the region to amplify and to facilitate the different steps of this process.

Our objectives are:
1. Produce mutagenized populations of japonica (cv. Nipponbare) and indica (cv. IR64) rice
2. Identify mutations in a set of stress tolerance candidate genes.
3. Provide TILLING services and mutant stocks to the rice functional genomics community, depositing the TILLed lines at the GSOR (Dale Bumpers National Rice Research Center, Stuttgart, AK) and the data in the Gramene database.
4. Provide training workshops to scientists interested in applying TILLING to crop plants.

For TILLING purposes, rice has been recalcitrant to mutagenesis and multiple attempts have previously failed to produce the required density of mutations. Recently, we have made progress. The Tai laboratory has identified a mutagenesis protocol for Nipponbare that produced a satisfactory mutations rate for a TILLING service (≥1/400 kb DNA) based on a pilot analysis of 768 M2 individuals. A total of 2000 M2 lines are available for a TILLING service and a scale up is in progress to produce 5,000 or more M2 Nipponbare lines. We anticipate that TILLING with these resources will be publicly available using an existing, user-friendly web interface (http://tilling.fhcrc.org:9366/) coupled with distribution of the TILLED stock from the Dale Bumpers Center. Leung's lab at IRRI has produced a high-dosage EMS (2%) population of IR64 with an estimated mutation density of 1.6/Mb. They are testing a "sequential mutagenesis" strategy to increase mutation density. IRRI is also using conventional agarose gel electrophoresis for simplified detection of CEL1-cleaved products using. This method alleviates the need for labeled primers, is suitable for developing countries, and it could enable TILLING of larger amplicons (2-3 kb), potentially raising the efficiency of mutation discovery.

The project represents collaboration between the laboratories of Luca Comai, Steve Henikoff, Hei Leung and Thomas Tai. The high throughput TILLING facility in Seattle is staffed by Brad Till, Jennifer Cooper, Rob Laport et al.. The bioinformatics support in Seattle is by Elizabeth Greene, Troy Zerr, and Jorja Henikoff. Japonica rice mutant population development is by Thomas Tai laboratory and Peter Colowit in Davis. Work on indica rice at IRRI involves Fulin Qiu, Chitra Raghavan, Janli Wu, and Ken McNally. Outside collaborators included Nori Kurata from Japan.

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# INTERNATIONAL RICE FUNCTIONAL GENOMICS CONSORTIUM

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In 2000, IRRI initiated an International Working Group on Rice Functional Genomics to bring diverse research groups together to discuss mutual interests and collaboration that would benefit from the rice genome sequencing effort. This working group has evolved into a more structured International Rice Functional Genomics Consortium (IRFGC) in January 2003 to:

- facilitate communication and exchange of resources and information
- develop collective goals and collaborative projects
- accelerate delivery of research products to benefit rice production
- leverage support from governmental and international funding agencies

An interim steering committee was formed to develop a basic framework and working principles for the consortium (http://www.iris.irri.org/IRFGC). The initial committee composed of 21 members from 17 institutions of 12 countries. A unique feature of IRFGC is its emphasis on the practical aspects of plant improvement and on mobilizing diverse expertise and experience from rice research and breeding institutions around the world. The IRFGC is in essence a "federally" coordinated collaborative network with a long-term goal of determining the function all rice genes, focusing on:

- Critical genes and gene networks important to agricultural improvement
- What genes make *indica* different from *japonica?*
- What genes make the other AA genomes different from *japonica* and *indica?*
- What genes make rice different from other monocots and *Arabidopsis*?

Since its formation, the IRFGC has leveraged support from research centers with long-term commitment to rice research and projects supported by national and international programs. It is noteworthy that of the 13 rice functional genomics projects supported by USDA NRI since 2003, seven are directly linked with IRFGC. In 2005, the initiatives coordinated by IRFGC include:

- Rice SNP project (supported by USDA NRI, Generation Challenge Program, and IRRI)
- Rice mutant phenotyping network (supported by Generation Challenge Program)
- A set of projects focusing on biotic and abiotic stresses (supported by the USAID Linkage Program and IRRI).

The IRFGC is also responsible for organizing the annual International Rice Functional Genomics Symposium (Shanghai 2003, Tucson 2004, Manila 2005, and Montpellier 2006).

At the recently concluded Rice Functional Genomics Symposia held in Manila, it was suggested that a small core committee should be formed to advocate overall consortium objectives whereas several subcommittees can be organized to coordinate specific activities and to involve the research community widely. It was also proposed that a systematic approach is necessary to identify the basic function of all rice genes, and that detailed discussion involving the community is needed to develop a road map with quantifiable progress and time line. Therefore the USDA NRI and RiceCAP meetings provide a timely opportunity to interact with various USDA projects to contribute to this discussion.

Acknowledgment: The IRFGC is supported by multiple funding sources and institutions, but most importantly by individuals willing to contribute their time to organize events and to subscribe to the consortium approach to advance the overall goals.